



PATENT
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IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: ATABEKOV, Joseph et al. Conf.: 5590
Appl. No.: 09/911,732 Group: 1636
Filed: July 25, 2001 Examiner: Lambertson, David
For: METHOD FOR COEXPRESSION OF MORE THAN
ONE GENE IN EUKARYOTIC CELLS

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The undersigned hereby declare the following.

- 1) We are the inventors of the subjected matter disclosed in the above-captioned application. As such, we fully understand the invention and field of the art surrounding IRES sequences.
- 2) We have reviewed the outstanding office action and understand that the Examiner has concerns about certain issues pertaining to the invention.
- 3) Specifically, we understand that the Examiner questions the following two points, which we will address in turn.

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a) As a first point, the Examiner has reviewed the journal article Ivanov et al., *Virology*, 232:32-43(1997). The Examiner has noted that the Ivanov et al. article suggests that the IRES sequence from crTMV is completely unique and that corresponding IRES sequences have not been found even in other tobamoviruses.

b) As a second point, the Examiner has requested a showing of why the disclosed IRES sequences in the specification support that the inventors were in possession of the entire genus of IRES sequences from tobamoviruses at the time of filing of the application, i.e in May of 1997.

Each of points a) and b) are addressed below.

4) Point a) - The teachings of Ivanov et al.

The Examiner noted upon review of the article of Ivanov et al. that the reference appears to teach that the IRES sequence from crTMV is completely unique and other tobamoviruses do not have corresponding IRES sequences.

The undersigned would like to initially point out that with the exception of Timo Korpela all of the present inventors are also authors on the Ivanov et al. article. As such, we have first hand knowledge of the experiments, results and conclusions presented in Ivanov et al. Essentially, the conclusions reported in Ivanov et al. are reflective of studies conducted prior to the present invention. Ivanov et al. was submitted as a manuscript in August

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of 1996, before the Finnish priority date of the present application of May 30, 1997. Subsequent to the submission of the Ivanov et al. manuscript we determined that certain conclusions that had been reached were, in fact, incorrect.

In Ivanov et al., wheat germ extracts (WGE) of rabbit reticulocyte lysates (RRL) *in vitro* translation methods were used in the experiments to detect TMV U1 IRES sequences upstream from the coat protein gene as shown in shown in Fig. 7B and 7C of Ivanov et al. However, we later realized that IRES activity of the sequence upstream of the coat protein gene of TMV U1 cannot be detected by the wheat germ extract (WGE) of rabbit reticulocyte lysate (RRL) *in vitro* translation methods used in the experiments.

Since the corresponding sequence of the crucifer-infecting TMV (cr-TMV) showed IRES activity in similar experiments, we had erroneously concluded, at the time of the work reported in Ivanov et al., that cr-TMV was unique for IRES elements.

However, we later determined in experiments that led to the present application, that the failure to detect the IRES sequences in TMV U1 was an experimental artifact and we detected IRES activity of the sequence upstream of the coat protein gene of TMV U1 in yeast cells. It thus turned out that the conclusion of the Ivanov et al. reference that cr-TMV was unique in having IRES elements was incorrect.

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5) Point b) Support for the genus of tobamoviruses

According to information from the International Committee on Taxonomy of Viruses (ICTV), see: www.ncbi.nlm.nih.gov/ICTVdb/lactv, the genus *Tobamovirus* presently has 19 species. We estimate that at the time when the Finnish priority application FI 972293 (filing date: May 30, 1997) was filed, there were less than 10 species of the genus *Tobamovirus* known. We further note that taxonomic classification of a virus requires that the genome sequence of the virus to be classified is at least partially known.

In our opinion, the disclosure in the specification of IRES sequences from three difference tobamoviruses would lead one skilled in the art, i.e. the typical scientist in the field of virology, to conclude that we had sufficiently described the entire genus of tobamovirus IRES sequences. It is evident from the specification, including the drawings, that IRES sequences from tobamoviruses possess certain common structural features, which one would expect would likely be in any tobamovirus IRES sequence.

For example, Figures 1B to 1H of the above-captioned application show predicted secondary structures of various tobamoviral IRESSs. The 3'-end of the IRESSs is defined by the AUG start codon. The RNA sequences forming these IRESSs have pronounced self-complementarity, enabling the formation of multiple duplex (base-paired) structures in major parts of the IRES RNA sequences. Major parts of the IRESSs are involved in secondary structures. The

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IRESs form at least one, and typically two to four stem-loops.

As can be further seen from the figures, the stems of the stem-loops can be very large, whereby large stems may be interrupted by bulges. These bulges frequently occur as symmetric bulge loops, i.e. the bulges occur on opposing strand segments and opposing bulges of a bulge loop frequently have the same number of bases. In addition, the primary structures of the IRESs share the common feature of being U-rich.

Additionally, coat protein IRESs are typically rich in purine bases in loops that connect stem-loops structures. Such purine-rich loops that connect stem-loop structures typically have more than four bases.

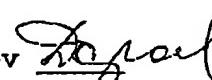
The skilled person envisions that tobamoviral IRESs other than those specifically mentioned and shown in Fig. 1B to 1H would have the same structural properties as described above under item 1 and, as a consequence, will also have IRES activity. As such, the claimed genus of IRES sequences from tobamoviruses for the present invention is sufficiently described by the recognition of common structural features.

We each hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

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statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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By Yuri Dorokhov  Date 28.09.2004

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